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Host-selection behavior and physiological mechanisms of the cotton aphid, *Aphis gossypii*, in response to rising atmospheric carbon dioxide levels

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ABSTRACT

Rising atmospheric carbon dioxide (CO_2) levels can markedly affect the growth, development, reproduction and behavior of herbivorous insects, mainly by changing the primary and secondary metabolites of their host plants. However, little is known about the host-selection behavior and the respective intrinsic mechanism of sap-sucking insects in response to elevated CO_2. In this experiment, the host-selection behavior, as well as the physiological mechanism based on the analysis of growth, development and energy substances, and the expression of the olfactory-related genes of the cotton aphid, *Aphis gossypii*, were studied under ambient (407.0 ± 4.3 μl/L) and elevated (810.5 ± 7.2 μl/L) CO_2. The results indicated that the aphids reared under ambient and elevated CO_2 did not differ in their level of preference for cotton seedlings, whatever the CO_2 conditions in which the plants developed. However, aphids reared under elevated CO_2 showed a greater ability to respond to the plant volatiles compared to aphids that developed under ambient CO_2 (+23.3%). This suggests that rising atmospheric CO_2 enhances the activity of host selection in this aphid. Compared with ambient CO_2, elevated CO_2 significantly increased aphid body weight (+36.7%) and the contents of glycogen (+18.9%), body fat (+14.6%), and amino acids (+16.8%) and increased the expression of odor-binding protein genes, *OBP2* (+299.6%) and *OBP7* (+47.4%), and chemosensory protein genes, *CSP4* (+265.3%) and *CSP6* (+50.9%), potentially enhancing the overall life activities and upregulating the olfactory ability of *A. gossypii*. We speculated that the rising atmospheric CO_2 level would likely aggravate the damage caused by *A. gossypii* due to the higher potential host selection and increased general activity under future climate change.

1. Introduction

Rising atmospheric carbon dioxide (CO_2) levels are one of the most concerning issues about global climate change. CO_2 has continuously risen from 280 ppm before the industrial revolution to 402 ppm at present (Mauna Loa Observatory: NOAA-ESRL), and is expected to reach at least 550 ppm by the middle of the 21st century (Pachauri et al., 2014) and 800 ppm by the turn of this century (Mastrandrea et al., 2011). As an important constituent for plant photosynthesis, especially for C_3 plants, the change in atmospheric CO_2 concentrations directly affects plant photosynthesis and growth (Bazzaz and Catovsky, 2002; Leakey et al., 2009). Elevated CO_2 also indirectly affects the growth and development of herbivorous insects and their physiological metabolism by altering plant biomass and quality, including changes in the composition and content of chemical substances in their host plant tissues (Stacey and Fellowes, 2002; Chen et al., 2004, 2006; Ge and Chen, 2006; Jiang et al., 2016). Because insects account for about half of all living things on the planet and are highly sensitive to global climate change (such as elevated CO_2), the steady increase in environmental CO_2 will likely have a significant negative impact on the maintenance of ecosystem structure and function (Ge, 2011).

Insects and plants have formed complex interrelationships through long evolution, and the breeding of insect populations depends largely on finding suitable host plants and getting appropriate and adequate nutrition (Fan et al., 2014). Sensitive olfactory systems allow insects to accurately perceive chemical signals in the environment (Field et al., 2000). The olfactory receptors of insects are located on the antenna and maxillary palpi; both organs have olfactory receptor neurons (ORNs), which are covered with different types of olfactory receptors (Hallberg, 1982; Sukontason et al., 2007; Liu et al., 2011; Wang et al., 2012). The odor molecules enter the antennal lymph through micropores in the epidermis of the olfactory receptors, and with the help of related

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proteins pass through the hydrophobic lymph to the ORNs where they then produce nerve impulses by the effects of ORN odorant receptors. Different proteins carry different types of odors to complete the insects' initial identification of the host plant (Xia et al., 2008). During this period, the participation of many proteins such as odorant-binding proteins (OBPs), chemosensory proteins (CSPs), odorant degrading enzymes (ODEs), and sensory neuron membrane proteins (SNMPs) are required, and the OBPs and CSPs are the primary peripheral olfactory proteins that play critical roles in odor detection (Zhao et al., 2017). The expression of odorant-binding protein genes (including *OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (including *CSP4* and *CSP6*) are highly expressed in the head of *A. gossypii* (Gu et al., 2013; Xu, 2014; Zhao et al., 2017). Insects need additional basic energy substances during host selection, so they gain energy substances and maintain normal biochemical conditions through breathing. Various energy substances, such as glycogen, fats, and amino acids, produce the necessary energy for insects' life activities according to specific metabolic pathways (Rankin and Burchsted, 1992).

Many experiments have been carried out to study the host-selection behavior of insects. Liu et al. (2002) and Chen et al. (2005) both used leaf selection methods and electropenetrography (EPG) technology to study host selection and specialization in the cotton aphid, *Aphis gossypii*. Wang et al. (2017) used the Petri dish selection method to study the host selection of two kinds of specialized aphids. Geier and Boeckh (1999) used a Y-type tube olfactory to study the host selection of *Aedes aegypti* on a human hand, an extract with human skin residues, L-(C)-lactic acid and CO_2; they found that CO_2 stimulated the flight of *A. aegypti*, and it was more obvious after coordination with lactic acid. Reddy et al. (2004) used a wind tunnel to study the host plant-mediated orientation and oviposition of *Plutella xylostella* on four different brassica host plants. And Rim et al. (2017) used a Y-type tube olfactory to study whether the plant-infestation experience of *Nesidiocoris tenuis* affected its subsequent prey-finding behavior. Moreover, numerous studies confirm that high atmospheric CO_2 concentrations can affect the growth, reproduction, feeding behavior and oviposition of some insects (Guerenstein and Hildebrand, 2008; Sun et al., 2011; Couture et al., 2012). As a phloem-feeding insect, the cotton aphid is one of the most important agricultural pests of cotton production worldwide (Castle et al., 1992; Weathersbee and Hardee, 1994; Birkett and Pickett, 2003). However, the influence of rising atmospheric CO_2 levels on the host-selection behavior of the cotton aphid is not well documented.

In this study, the host-selection behavior, as well as the physiological mechanism based on the analysis of growth, development and energy substances, and the expression of the olfactory-related genes of the cotton aphid were studied under ambient and elevated CO_2. The objectives of this study were to: 1) examine the host-selection behavior of *A. gossypii* as influenced by elevated CO_2 using an olfactometer, 2) quantify the parameters of insect growth and development and accumulated energy substances in insect bodies as a proxy for host-selection behavior influenced by two CO_2 levels, and 3) investigate the expression of odorant-binding protein genes (including *OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (including *CSP4* and *CSP6*) to elucidate the molecular mechanism in the changes in host-selection behavior of *A. gossypii* in response to rising atmospheric CO_2 levels.

2. Materials and methods

2.1. Setup of CO_2 levels

This study was conducted in electronically controlled growth incubators (GDN-400D-4/CO_2; Ningbo Southeast Instrument CO., LTD., Ningbo, China) with a gas-tank system to supply 99% pure CO_2 gas and maintain the desired CO_2 concentrations. In these growth incubators, the environmental conditions were set at 27 °C and 70% RH during the day and 26.5 °C and 70% RH at night. The photoperiod was L14:D10 and the light intensity was 20,000 l× in each growth incubator. Two levels of CO_2 concentration were applied continuously: ambient CO_2 (401.2–411.2 μl/L; mean: 407.0 ± 4.3 μl/L), representing the current level of atmospheric CO_2 concentration, and elevated CO_2 (803.0–818.6 μl/L; mean: 810.5 ± 7.2 μl/L), simulating the predicted level of atmospheric CO_2 concentration at the end of this century (Mastrandrea et al., 2011). Four growth incubators were used as replicates for each CO_2 treatment.

2.2. Host plants and insect colony

The cotton cultivar (cv. C111) was supplied by the Jiangsu Academy of Agricultural Sciences and planted in plastic pots (12 cm in diameter and 15 cm high) filled with nutritional soil (Xingnong Organic Fertilizer CO., LTD., Zhenjiang, China) in the electronically controlled growth incubators. After the emergence of seedlings, the cotton plants were thinned to one plant per pot and exposed to the ambient and elevated CO_2 levels. Potted plants were watered moderately every other day, and no additional chemical fertilizers or insecticides were used throughout the experiment. The experiment consisted of twenty-four plastic pots (i.e., 24 cotton plants) placed in each growth incubator, with a total of 192 cotton plants (24 plants per growth incubator × 4 growth incubators per CO_2 level × 2 CO_2 levels) for the entire experiment. The experimental pots (plants) in each growth incubator were re-randomized once a week to minimize positional effects within the incubator. Thirty days after germination, each experimental cotton seedling (192 total plants) was inoculated with cotton aphids for host-selection behavior studies.

The apterous *A. gossypii* used in this study were provided by the Insect Ecology Group of the Department of Entomology, College of Plant Protection, Nanjing Agricultural University. A single apterous aphid was randomly selected and reared on 30- to 50-day-old cotton (cv. C111) seedlings in electronically controlled growth chambers to establish a standardized colony.

2.3. Growth and accumulation of energy substances by A. gossypii adults

In each growth incubator of ambient and elevated CO_2, 200 newly emerged *A. gossypii* adults (a total of 200 individuals per growth incubator × 4 growth incubators per CO_2 level × 2 CO_2 levels = 1600 newly emerged adults) were randomly selected from the above aphid colony and inoculated singly onto a fully expanded leaf in glass Petri dishes (150 mm diameter; 10 individuals per dish and 20 dishes per growth incubator). Cotton leaves were excised from the 30- to 50-day-old cotton seedlings, and a single aphid was inoculated into each Petri dish and allowed to oviposit for 12 h. Then, all the inoculated aphid adults were removed and all the offspring were reared to adulthood for the following experiments. Forty newborn offspring (10 individuals per growth incubator × 4 growth incubators) in each CO_2 treatment were reared individually until each aphid nymph reached adulthood, and the total nymphal duration for each individual aphid was recorded. Two hundred newly emerged adult aphids (50 individuals per growth incubator × 4 growth incubators) in each CO_2 treatment were randomly selected and divided into 20 replicates (10 adults per replicate) to measure the body weights of cotton aphids using an electronic microbalance with an accuracy of ± 1 μg (Mettler Toledo XP6, Switzerland).

Three types of energy substances, including glycogen, body fat and amino acids, were also measured in the adult aphids. Here, another 60 adult aphids (15 individuals per growth incubator × 4 growth incubators) were randomly selected from each CO_2 treatment and equally divided into three replicates for weighing using the same electronic microbalance; these were ground into a homogenate with 10% trichloroacetic acid, incubated for 3 h at 4–5 °C, and this mixture was then centrifuged at 6000 rpm for 10 min. The supernatant was transferred and 95% ethanol and a drop of saturated Na_2SO_4 were added. The mixture was incubated overnight at 5 °C and centrifuged at 3000 rpm for 20 min. The supernatant was removed and precipitated in the

centrifuge tube for 30 min, and then dissolved in distilled water; this was used to determine the glycogen content in the test aphids using the anthrone method (Yi et al., 2009). In addition, 300 newly emerged adult aphids (75 individuals per growth incubator × 4 growth incubators) were randomly selected from each CO_2 treatment and equally divided into 3 replicates (100 individuals per replicate). These were then weighed using the same electronic microbalance and dried for 48 h using a Christ freeze-dryer (Christ ALPHA 2–4 LD plus; Martin Christ CO. LTD., Osterode, Germany) to determine the constant dry weight (DW). These dried aphids were ground into a homogenate with a mixture of chloroform and methanol (chloroform:methanol = 2:1), the suspension was centrifuged for 10 min at 12000 rpm and the supernatant was removed. This step was repeated once more and the precipitate was then dried to a constant weight (LDW), and the body fat measurement was calculated as DW minus LDW (Colinet et al., 2007). Another 900 newly emerged adults (300 individuals per growth incubator × 4 growth incubators) were randomly selected from each CO_2 treatment and were equally divided into 3 replicates (100 individuals per replicate). These were weighed, dried to a constant weight using the same Christ freeze-dryer, and the hydrochloric hydrolysis method was used for the pretreatment of samples (Sun et al., 2008). The samples were then further processed to quantify the amino acids using the automatic amino acid analyzer (L-8900; Hitachi High-Technologies Corporation, Tokyo, Japan). The glycogen, body fat, and amino acid contents were calculated as μg/mg of fresh weight.

2.4. Measurement of the host-selection behavior of A. gossypii adults

The host-selection behavior of *A. gossypii* adults as influenced by CO_2 level was quantified using a four-chamber olfactometer (PSM4-150; Nanjing Pusen Instrument CO. LTD., Nanjing, China). Each chamber received three 40-day-old cotton seedlings grown under ambient CO_2 (aCotton), elevated CO_2 (eCotton), or ambient air as a control, while the fourth arm was sealed (Fig. 1). In this experiment, an 8 W fluorescent lamp was placed above the four-arm motherboard and the flow meter was adjusted to deliver a consistent airflow of 3 L/min to all three sides. Twenty newly emerged adults from each growth incubator (a total of 80 individuals from 4 growth incubators) were selected from each CO_2 treatment and starved for 2 h, and then released to the center of the four-arm motherboard to observe their host-selection behavior. If the sampled adults reached the nesting area of one arm (Fig. 1) within 6 min, the treatment (*a*Cotton, *e*Cotton, or control) corresponding to that arm was considered as the choice of the released aphids. Test insects that did not reach any nesting area within 6 min of release were considered non-responders (i.e., no choice). To avoid biases in the behavioral observations between tests, the air compressor was turned off for 10 min and wiped with anhydrous alcohol after each test. The intake pipe was also exchanged after each test, and all tests were carried out in a clean, uniform, well-ventilated and relatively closed laboratory.

2.5. Bioassay of expression levels of the odor-binding protein genes and chemosensory protein genes in A. gossypii adults

The expression of the odor-binding protein (*OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*) in adult *A. gossypii* fed on cotton seedlings grown under ambient and elevated CO_2 were assayed through reverse transcription and real-time PCR analyses.

2.5.1. RNA preparation and reverse transcription

A set of 20 newly emerged adult *A. gossypii* from each growth incubator were randomly collected from each CO_2 treatment. Aphids collected from each incubator served as one biological replicate (a total of 4 biological replicates for each CO_2 treatment). The total RNA was extracted from each replicate sample using the TRIzol® reagent (Invitrogen). The concentration and quality of the samples were determined by a NanoDrop™ spectrophotometer (Thermo Scientific) and 1.5% agarose gel electrophoresis. The 1st strand complementary cDNA templates were synthesized with 100 ng of total RNA by using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan). Reverse transcriptase reactions were performed in a final volume of 20 μl.

2.5.2. Real-time PCR analysis

Each cDNA product was diluted twice from 20% to 1.25% solution using RNase-free dH_2O to bring the Ct value within the suitable range of 15–35 based on preliminary experiments. For the fluorescence-based quantitative real-time PCR (qRT-PCR), 2 μl cDNA dilution and 0.2 μM primer were used in 1 × SYBR® *Premix Ex Taq*™ (TaKaRa, Japan) with the 7500 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA) following the supplier's instructions. The reactions were performed in a final volume of 20 μl. This experiment was performed at different concentration of CO_2, thus, it was necessary to use the geNorm algorithm to analyze the stability of potential housekeeping genes (*GAPDH*, *18S*, *RPL7*, *EF1α*, *HSP70* and *β-actin*) of the different samples. The assessment criteria was M < 1.0 (Etschmann et al., 2006). Ultimately, *RPL7* gene was selected as the reference gene in this study (Ma et al., 2016). Then, specific primers were designed using Beacon Designer™ 7.9 software, and the housekeeping gene *RPL7* (Ma et al., 2016) was used as the internal standard to analyze the expression levels of the target genes, including the odor-binding protein genes (*OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*). All primers for qRT-PCR analysis are shown in Table 1. Quantification of the transcript levels of the target genes was conducted following the $2^{-\Delta\Delta Ct}$ normalization method (Livak and Schmittgen, 2001). The expression levels of the internal control gene (i.e., *RPL7*) were examined in every PCR plate to eliminate systematic errors. For each biological replicate

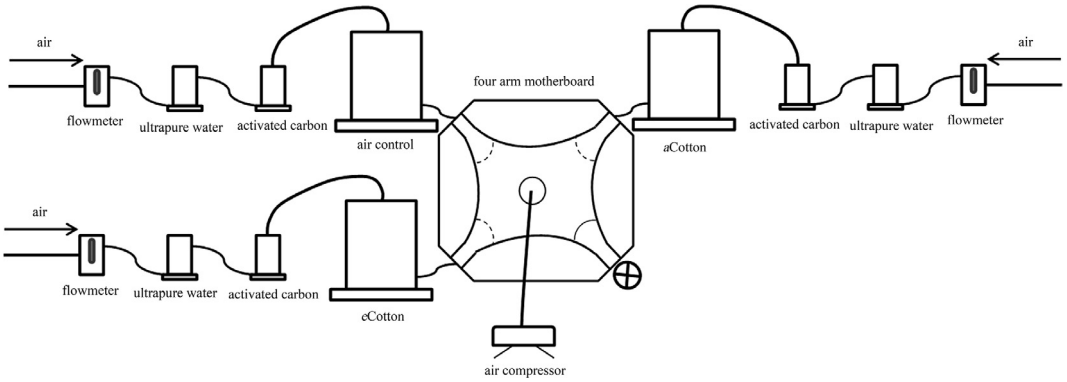

Fig. 1. An olfactometer with a four-arm motherboard (length:width:height = 35 cm:29 cm:4.3 cm) used to measure the host-selection behavior of the cotton aphid, *Aphis gossypii*. Note: The angle between two adjacent arms is 90°; ⊕ indicates that the arm was sealed; *a*Cotton and *e*Cotton are the cotton seedlings grown under ambient and elevated CO_2, respectively).

Table 1
Primers designed and used in measuring the transcript expression levels of the odor-binding protein genes (*OBP2*, *OBP6* and *OBP7*), chemosensory protein genes (*CSP4* and *CSP6*), and the housekeeping gene (i.e., *RPL7*) of *A. gossypii* in qRT-PCR experiments.

Primers	Sequences (5′ to 3′)	GenBank Accession	Description
OBP2	Forward: CACGGAGCGAACAACTG Reverse: CCATCGTCCACACTGAAC	KC161555.1	Odorant-binding protein gene
OBP6	Forward: TGCGATCATCTGCCAAACA Reverse: AGAGAGCTCGGCATTCATTATC	KC161559.1	
OBP7	Forward: CCGAGAACAACAACAACATA Reverse: GCCAACATCGTCATCTTG	KC161560.1	
CSP4	Forward: CCAGAATTGCAGTAGTCTGTGT Reverse: TGTGGTCGTATTTGGTAGTGTAAG	KC161566.1	Chemosensory protein gene
CSP6	Forward: CGTCTCTATAACTATGACTGTG Reverse: TCTTCGCCTTCTGGTGTA	KC161568.1	
RPL7	Forward: TGCCGGAGTCTGTACTCAA Reverse: TCACACCACGAATACGCA	KP676382	Housekeeping gene

(four replicates per CO_2 treatment), three technical repeats were performed in qRT-PCR analysis.

2.6. Data analysis

The measured indexes, including nymphal duration, adult body weight, and the content of energy substances (glycogen, body fat, and amino acids) and the relative transcript levels of the target genes [odorant-binding protein (*OBP2*, *OBP6*, and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*)] were analyzed by one-way analysis of variance (ANOVA) with two CO_2 levels (ambient CO_2 vs. elevated CO_2) as the source of variability (SPSS v.20.0; IBM Corporation, Armonk, NY, USA). One-way ANOVA was also used to analyze the olfactory response to the three odor sources of *A. gossypii* reared under two CO_2 conditions. Significant differences in the measured indexes between the treatments were analyzed by the LSD test at $P < 0.05$.

3. Results

3.1. Effects of CO_2-modulated cotton seedlings on the host-selection behavior of A. gossypii

A. gossypii adults significantly preferred to select cotton seedlings regardless of the CO_2-rearing levels (ambient CO_2-reared plants, *a*Aphid: +109.09%; *e*Aphid: +170.00% and elevated CO_2-reared plants, *a*Aphid: +136.36%; *e*Aphid: +270.00%) in contrast to the air control treatment ($P < 0.05$; Fig. 2). The aphids reared under ambient and elevated CO_2 did not differ in their level of preference for cotton seedlings, whatever the CO_2 conditions in which the plants developed. However, the number of adult aphids preferred cotton seedlings grown under elevated CO_2 (*e*Cotton) was higher than that of adult aphids preferred cotton seedlings grown under ambient CO_2 (*a*Cotton) for both the ambient CO_2 reared aphids (+13.04%) and elevated CO_2 reared aphids (+37.04%) ($P > 0.05$; Fig. 2).

At the same time, elevated CO_2-reared aphids were significantly more responsive to host odor compared to ambient CO_2-reared aphids and this phenomenon was consistent across all three host odor sources (*a*Cotton, *e*Cotton, and air control) ($P = 0.004 < 0.01$; Fig. 3). Averaged across three odor sources, the number of responding *e*Aphids was significantly higher (+23.3%) than that of responding *a*Aphids ($P < 0.05$; Fig. 3).

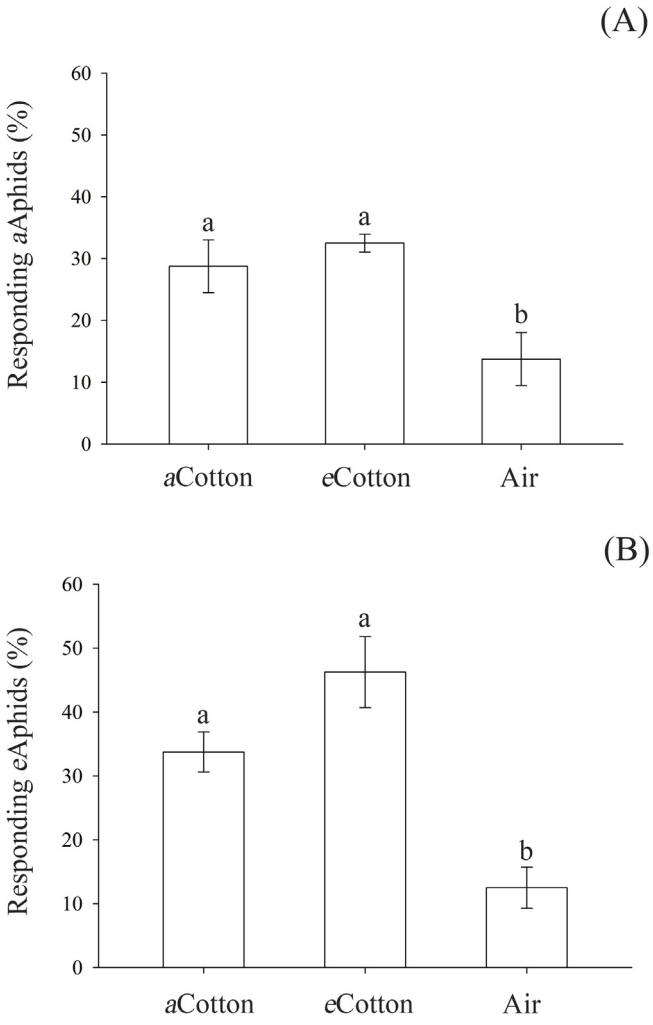

Fig. 2. The percentage of adult *A. gossypii* reared under ambient CO_2 (i.e., *a*Aphids; A) and elevated CO_2 (i.e., *e*Aphid; B) responding to the odor source emanating from cotton seedlings grown under two CO_2 conditions versus an air control deployed in a four-arm olfactometer (Note: each value represents the average (± SE). Aphids – *a*Aphid and *e*Aphid correspond to sampled adult aphids fed on the excised fully expanded leaves from the cotton seedlings grown under ambient and elevated CO_2, respectively; Hosts – *a*Cotton and *e*Cotton correspond to sampled cotton seedlings grown under ambient and elevated CO_2, respectively; Air – the air control treatment; Different lowercase letters indicate significant differences among the host treatments (*a*Cotton, *e*Cotton, and Air) within the same CO_2-reared aphids by the LSD test at $P < 0.05$).

3.2. Effects of CO_2 levels on the growth and energy substances of A. gossypii adults

CO_2 levels significantly affected the adult body weight ($F = 39.49$, $P < 0.001$), and the contents of glycogen ($F = 14.17$, $P = 0.019 < 0.05$), body fat ($F = 10.24$, $P = 0.033 < 0.05$), and amino acids ($F = 129.64$, $P < 0.001$) of the sampled adult aphids fed on cotton grown under ambient and elevated CO_2 (Fig. 4). Compared with ambient CO_2, elevated CO_2 significantly ($P < 0.05$) increased adult body weight (+36.66%; Fig. 4B) and the contents of glycogen (+18.90%; Fig. 4C), body fat (+14.56%; Fig. 4D) and amino acids (+16.78%; Fig. 4E) in *A. gossypii* (see Table 2).

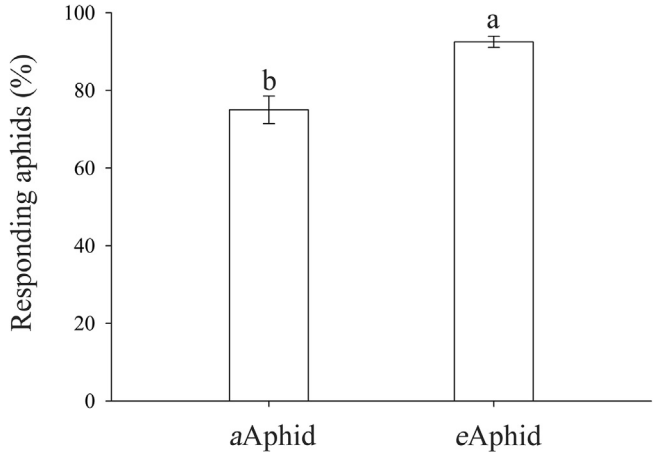

Fig. 3. The percentage of respondent *a*Aphid versus *e*Aphid that were exposed to the three odor sources in an olfactory choice study (Note: each value represents the average (± SE). *a*Aphid and *e*Aphid – the sampled adult aphids fed on the excised fully expanded leaves from the cotton seedlings grown under ambient and elevated CO_2, respectively; Different lowercase letters indicate significant differences between the sampled adult aphids by the LSD test at $P < 0.05$).

3.3. Effects of CO_2 level on the expression levels of odorant-binding protein genes and chemosensory protein genes in A. gossypii adults fed on cotton seedlings grown under ambient and elevated CO_2.

CO_2 levels significantly affected the expression levels of odorant-binding protein genes (*OBP2*: $F = 93.69$, $P < 0.001$; *OBP7*: $F = 11.36$, $P = 0.020 < 0.05$; Fig. 5A) and chemosensory protein genes (*CSP4*: $F = 448.48$, $P < 0.001$; *CSP6*: $F = 7.82$, $P = 0.038 < 0.05$; Fig. 5B) in *A. gossypii* adults fed on cotton seedlings grown under ambient and elevated CO_2. Compared with ambient CO_2, elevated CO_2 significantly ($P < 0.05$) increased the expression levels of *OBP2* (+299.58%; Fig. 3a), *OBP7* (+47.41%; Fig. 3a), *CSP4* (+265.34%; Fig. 3b), and *CSP6* (+50.94%; Fig. 3b).

4. Discussion

Herbivorous insects can identify volatile substances released by host plants during the host selection process through the olfactory receptor (Bartlet et al., 1993; Verkerk and Wright, 1994; Schoonhoven et al., 1998). Possell et al. (2005) detected a significant enhancement of isoprene emissions per unit leaf area in *Mucuna pruriens* under sub-ambient CO_2 concentrations (i.e., 180 µl/L) relative to ambient controls (i.e., 366 µl/L), but not for *Arundo donax*. Loreto et al. (2001) found that the overall emission of monoterpenes at elevated CO_2 will be inhibited because of a concurrent and strong down-regulation of monoterpene synthase activities. Vuorinen et al. (2004) found that volatile organic compound emissions that are induced by the leaf-chewing herbivores will not be influenced by elevated CO_2. This indicates that elevated CO_2 has specific effects on plant volatiles. However, the effects of atmospheric CO_2 concentrations on the volatiles of cotton plants have not been reported. In this study, the results indicated that the aphids reared under ambient and elevated CO_2 did not differ in their level of preference for cotton seedlings, whatever the CO_2 conditions in which the plants developed. However, there is a trend that a greater number of adult aphids preferred *e*Cotton than *a*Cotton for the responding *a*Aphids and *e*Aphid. Whether the future climate change with rising atmospheric CO_2 levels will affect the volatiles of cotton plants and lead to some changes in the host-selection behavior of *A. gossypii* needs further substantiation.

Elevated CO_2 inevitably alters plant metabolites, and this, in turn, would affect the performance of sap-sucking insects by the bottom-up effects of host plants in terms of nutritional status (Awmack and Leather, 2002). For example, elevated CO_2 significantly enhanced the foliar soluble matter of cotton plants, including soluble sugars, free amino acids and fatty acids, which has further positive effects on the population growth of *A. gossypii* in response to elevated CO_2 (Jiang et al., 2016). And the rising atmospheric CO_2 increases the population growth of *Acyrthosiphon pisum* by enhancing food ingestion and improving food quality plasticity, i.e., increasing the contents of amino acids and other nutrient components in host leaves and phloem saps (Guo et al., 2013). As a typical phloem-feeding insect, *A. gossypii* shows much more positive population growth under elevated CO_2 conditions than chewing insects and leaf-mining insects (Bezemer and Jones, 1998; Ge and Chen, 2006; Ge et al., 2010; Sun et al., 2015). In addition, Amsalem and Grozinger (2017) detected that elevated CO_2-treated queen of bumble bees were more active (particularly in terms of flight). In this study elevated CO_2 significantly increased adult body weight, which may provide more energy and favor the life activity of *A. gossypii*. Through oxidative metabolism energy materials such as glycogen, body fat, amino acids, and other constituents serve as primary energy substances in insects, which can use these energy substances singly or in combination as flight fuels (Rankin and Burchsted, 1992). Some short-distance flying insects, such as cockroaches and bees, can use sugars as energy materials (Elliott et al., 1984; Suarez et al., 2005). Body fat also plays an important role in the lifetime of insects. It is a body tissue that contains a variety of metabolic functions. One function is to store and release energy substances to respond to insect energy needs (Arrese and Soulages, 2010), and amino acids are generally used as a supplement for energy. Sun et al. (2008) found a significant increase in the amino acid content in *A. gossypii* under elevated CO_2. The results of this study showed that elevated CO_2 significantly increased adult body weight and the glycogen, body fat and amino acid contents in adult *A. gossypii* compared with ambient CO_2. Therefore, elevated CO_2 can improve the nutritional status of *A. gossypii*, which is more beneficial for the accumulation of energy substances in adult aphids and results in enhanced activity of cotton aphids toward the host crop under rising atmospheric CO_2 conditions.

Both the odorant-binding protein (OBP) and chemosensory protein (CSP) genes are believed to carry some functional proteins, which are involved in the initial identification of odor sensing, by capturing and transporting hydrophobic odor molecules through the hydrophobic lymph to the olfactory receptor neurons (Honson et al., 2005; Pelosi et al., 2005; Pelosi et al., 2006; Zhou, 2010; Sachse and Krieger, 2011). According to the qRT-PCR analysis, the expression levels of odorant-binding protein genes (*OBP2* and *OBP7*) in *A. gossypii* adults were significantly enhanced under elevated CO_2 compared with ambient CO_2. The combination of OBPs and odor molecules is the first biochemical reaction of herbivorous insects' specificity to identify the external odor substances and is the key component of the first function (You et al., 2012; Hu et al., 2013). Similarly, the chemosensory protein genes (*CSP4* and *CSP6*) in *A. gossypii* adults were also significantly improved under elevated CO_2 compared with ambient CO_2. The CSP genes bear more important functions than the OBP genes; one is to dissolve and transport different chemoreceptor fat-affinity ligands, and certain chemosensory proteins are involved in the functional part of olfaction (Nagnan-Le Meillour et al., 2000; Monteforti et al., 2002). Moreover, the CSP genes play an important role in sensory chemical stimulation (Steinbrecht et al., 1995; Ban et al., 2002; Pelosi et al., 2006). It can be presumed that elevated CO_2 can affect the expression of the partial OBP and CSP genes in adult aphids and then change their host-selection behavior at the molecular level. The peak expression of odorant-binding protein genes in different development duration of insects can be considered as a critical period for their physiological function in regulating insects' host-selection behavior (He et al., 2011; Li et al., 2013). In this study, the *OBP6* expression of *A. gossypii* adults did not differ between the *a*Aphids and *e*Aphids, indicating that the *OBP6* does not contribute to the regulation of the host-selection behavior during the adult stage

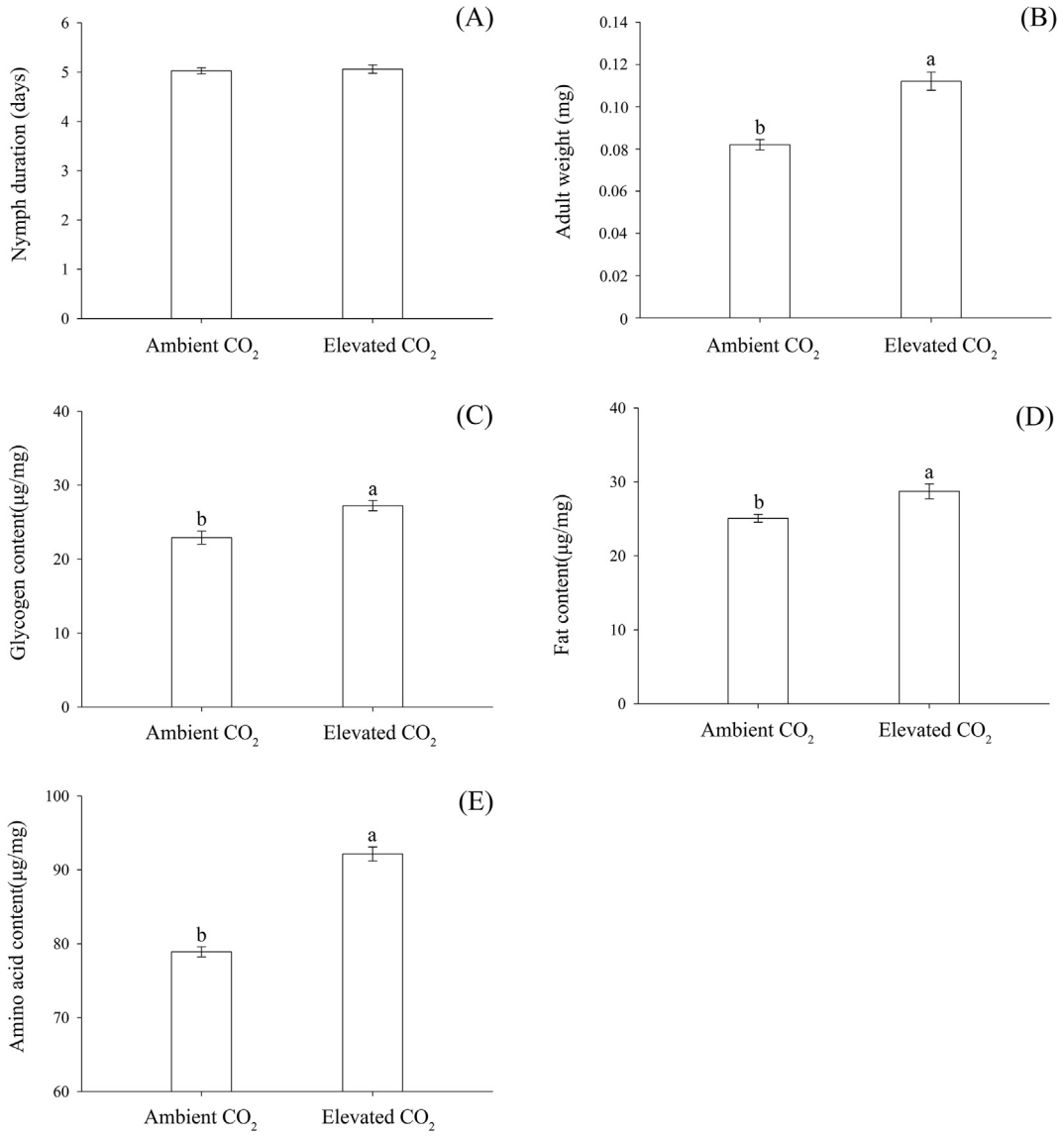

Fig. 4. Nymphal duration (A), adult body weight (B), and the contents of glycogen (C), body fat (D) and amino acids (E) of *A. gossypii* adults fed on cotton seedlings grown under ambient and elevated CO_2. (Note: each value represents the average (± SE). Different lowercase letters indicate significant differences between ambient and elevated CO_2 treatments by the LSD test at $P < 0.05$).

Table 2
One-way ANOVA for the effects of CO_2 levels (ambient vs. elevated) on the growth and development, energy substances, responding aphids, and the expression of odorant-binding protein (*OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*) of *A. gossypii* fed on cotton seedlings grown under ambient and elevated CO_2 (*F/P* values).

Measured indexes		*F* value	*P* value
Nymphal duration (days)		0.10	0.75
Adult body weight (mg)		39.49	< 0.001***
Adult body (μg/mg)	Glycogen	14.71	0.019*
	Fat	10.24	0.033*
	Amino acids	129.64	< 0.001***
Responding aphids (%)		21.00	0.004
Odorant-binding genes	*OBP2*	93.69	< 0.001***
	OBP6	0.19	0.68
	OBP7	11.36	0.020*
Chemosensory genes	*CSP4*	448.48	< 0.001***
	CSP6	7.82	0.038*

Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

regardless of CO_2 levels.

This study has attempted to elucidate the influence of climate change on the host-selection behavior of *A. gossypii*, that is, elevated CO_2 significantly increased responding aphids in comparison with ambient CO_2, indicating that the host selection activity of *A gossypii* adults can be enhanced under rising atmospheric CO_2 conditions. Two plausible reasons may be proposed for the enhanced host selection under elevated CO_2. First, the increased body weight and enhanced contents of energy substances can improve the general activity of *A. gossypii*. Second, elevated CO_2 increased the expression of odorant-binding protein (*OBP2* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*), which may further enhance the olfactory ability of *A. gossypii*. It is speculated that the rising atmospheric CO_2 level would likely aggravate the damage resulted by *A. gossypii* due to the higher potential host selection and increased general activity under future climate change.

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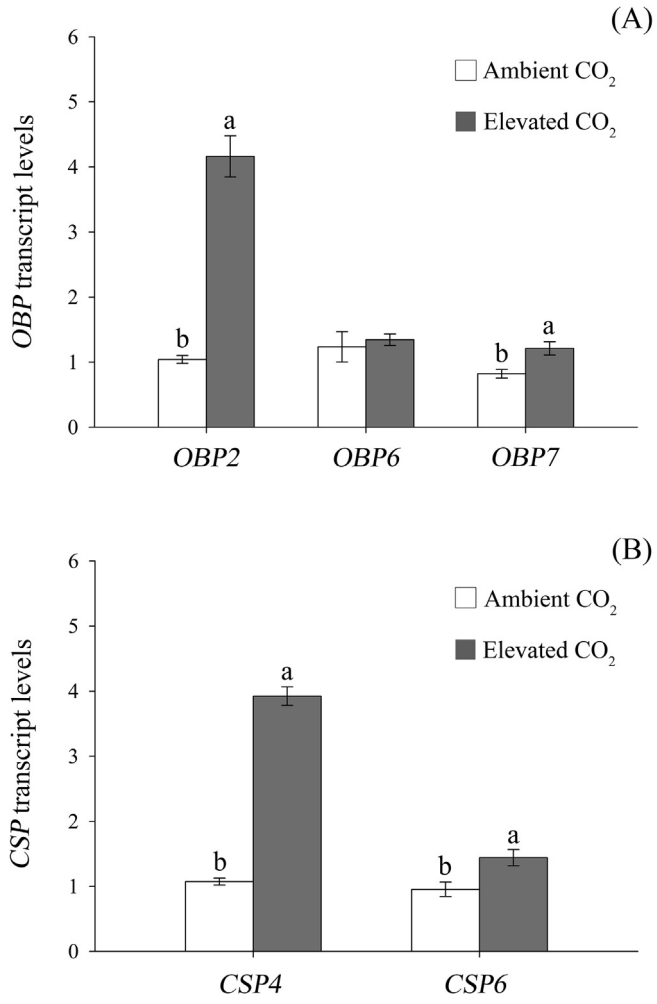

Fig. 5. Transcript expression of odorant-binding protein (A) and chemosensory protein genes (B) of *A. gossypii* adults fed on cotton seedlings grown under ambient and elevated CO_2 (Note: each value represents the average (± SE). Different lowercase letters indicate significant differences between ambient and elevated CO_2 by the LSD test at $P < 0.05$).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jinsphys.2018.05.011.

References

Amsalem, E., Grozinger, C., 2017. Evaluating the molecular, physiological and behavioral impacts of CO_2 narcosis in bumble bees (*Bombus impatiens*). J. Insect Physiol. 101, 57–65.
Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55, 207–225.
Awmack, C.S., Leather, S.R., 2002. Host plant quality and fecundity in herbivorous insects. Annu. Rev. Entomol. 47, 817–844.
Ban, L., Zhang, L., Yan, Y., Pelosi, P., 2002. Binding properties of a locust's chemosensory protein. Biochem. Biophys. Res. Commun. 293, 50–54.
Bartlet, E., Blight, M.M., Hick, A.J., Williams, I.H., 1993. The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape (Brassica napus) and to some volatile isothiocyanates. Entomol. Exper. Appl. 68, 295–302.
Bazzaz, F.A., Catovsky, S., 2002. Impact of global environmental change on plants: from cells to ecosystems. Encycl. Global Environ. Change 2, 94–111.
Bezemer, T.M., Jones, T.H., 1998. Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. Oikos 82, 212–222.
Birkett, M.A., Pickett, J.A., 2003. Aphid sex pheromones: from discovery to commercial production. Phytochemistry 62, 651–656.
Castle, S.J., Perring, T.M., Farrar, C.A., Kishaba, A.N., 1992. Field and laboratory transmission of watermelon mosaic virus 2 and zucchini yellow mosaic virus by various aphid species. Phytopathology 82, 235–240.
Chen, F.J., Ge, F., Liu, X.H., 2004. Responses of cotton to elevated CO_2 and the effects on cotton aphid occurrences. Acta Ecol. Sin. 24, 991–996.
Chen, F.J., Ge, F., Parajulee, M.N., 2005. Impact of elevated CO_2 on tri-trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. Environ. Entomol. 34, 37–46.
Chen, F.J., Wu, G., Ge, F., 2006. Responses of spring wheat to elevated CO_2 and their effects on *Sitobion avenae* aphid growth, development and reproduction. Chin. J. Appl. Ecol. 17, 91–96.
Colinet, H., Vernon, P., Hance, T., 2007. Does thermal-related plasticity in size and fat reserves influence supercooling abilities and cold-tolerance in *Aphidius colemani* (Hymenoptera: Aphidiinae) mummies? J. Therm. Biol 32, 374–382.
Couture, J.J., Meehan, T.D., Lindroth, R.L., 2012. Atmospheric change alters foliar quality of host trees and performance of two outbreak insect species. Oecologia 168, 863–876.
Elliott, J., Hill, L., Bailey, E., 1984. Changes in tissue carbohydrate content during flight of the fed and starved cockroach, *Periplaneta americana* L. Comp. Biochem. Physiol. A Physiol. 78, 163–165.
Etschmann, B., Wilcken, B., Stoevesand, K., von der Schulenburg, A., Sterner-Kock, A., 2006. Selection of reference genes for quantitative real-time PCR analysis in canine mammary tumors using the GeNorm algorithm. Vet. Pathol. 43, 934–942.
Fan, L.P., Wang, J.H., Yu, Z.J., Huang, F.Q., Kong, X.B., Wang, H.B., Zhang, S.F., Zhang, Z., 2014. Host selection behavior of *Micromelalopha sieversi* to five populus deltoides clones. Forest Research 27, 459–565.
Field, L.M., Pickett, J.A., Wadhams, L.J., 2000. Molecular studies in insect olfaction. Insect Mol. Biol. 9, 545–551.
Ge, F., 2011. Challenges facing entomologists in a changing global climate. Chin. J. Appl. Entomol. 48, 1117–1122.
Ge, F., Chen, F.J., 2006. Impacts of elevated CO_2 on insects. Acta Ecol. Sin. 26, 935–944.
Ge, F., Chen, F.J., Wu, G., Sun, Y.C., 2010. Research advance on the response of insects to elevated CO_2 in China. Chi. Bull. Entomol. 47, 229–235.
Geier, M., Boeckh, J., 1999. A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. Entomol. Exper. Appl. 92, 9–19.
Guerenstein, P.G., Hildebrand, J.G., 2008. Roles and effects of environmental carbon dioxide in insect life. Annu. Rev. Entomol. 53, 161–178.
Guo, H., Sun, Y., Li, Y., Tong, B., Harris, M., Zhu-Salzman, K., Ge, F., 2013. Pea aphid promotes amino acid metabolism both in *Medicago truncatula* and bacteriocytes to favor aphid population growth under elevated CO_2. Glob. Change Biol. 19, 3210–3223.
Gu, S.H., Wu, K.M., Guo, Y.Y., Field, L.M., Pickett, J.A., Zhang, Y.J., Zhou, J.J., 2013. Identification and expression profiling of odorant binding proteins and chemosensory proteins between two wingless morphs and a winged morph of the cotton aphid *Aphis gossypii* glover. PLoS One 8, e73524.
Hallberg, E., 1982. Sensory organs in lps typographus (Insecta: Coleoptera)—Fine structure of antennal sensilla. Protoplasma 111, 206–214.
He, P., Zhang, J., Liu, N.Y., Zhang, Y.N., Yang, K., Dong, S.L., 2011. Distinct expression profiles and different functions of odorant binding proteins in *Nilaparvata lugens* Stal. PLoS One 6, e28921.
Honson, N.S., Gong, Y., Plettner, E., 2005. Chapter nine–structure and function of insect odorant and pheromone-binding proteins (OBPs and PBPs) and chemosensory-specific proteins (CSPs). Recent Adv. Phytochem. 39, 227–268.
Hu, Y.Y., Xu, S.F., Wubie, A.J., Li, W., Guo, Z.B., Zhou, T., 2013. Research advance of olfactory proteins and olfactory mechanism in insects. Genom. Appl. Biol. 32, 667–676.
Jiang, S.L., Liu, T.J., Yu, F.L., Li, T., Parajulee, M.N., Zhang, L.M., Chen, F.J., 2016. Feeding behavioral response of cotton aphid, *Aphis gossypii*, to elevated CO_2: EPG test with leaf microstructure and leaf chemistry. Entomol. Exp. Appl. 160, 219–228.
Leakey, A.D., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R., 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J. Exp. Bot. 60, 2859–2876.
Liu, X.D., Zhang, L.J., Zhang, X.X., 2002. Studies on cotton aphid *Aphis gossygii* selectivity to host and its host-type. Acta Ecol. Sin. 22, 1281–1285.
Liu, Z., Hua, B.Z., Liu, L., 2011. Ultrastructure of the sensilla on larval antennae and mouthparts in the peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae). Micron 42, 478–483.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.
Li, Z.Q., Zhang, S., Luo, J.Y., Cui, J.J., Ma, Y., Dong, S.L., 2013. Two Minus-C odorant binding proteins from *Helicoverpa armigera*, display higher ligand binding affinity at acidic pH than neutral pH. J. Insect Physiol. 59, 263–272.
Loreto, F., Fischbach, R.J., Schnitzler, J.P., Ciccioli, P., Brancaleoni, E., Calfapietra, C., Seufert, G., 2001. Monoterpene emission and monoterpene synthase activities in the Mediterranean evergreen oak *Quercus ilex* L. grown at elevated CO_2 concentrations. Glob. Change Biol. 7, 709–717.
Ma, K.S., Li, F., Liang, P.Z., Chen, X.W., Liu, Y., Gao, X.W., 2016. Identification and Validation of Reference Genes for the Normalization of Gene Expression Data in qRT-PCR Analysis in *Aphis gossypii* (Hemiptera: Aphididae). J. Insect Sci. 16, 17.
Mastrandrea, M.D., Mach, K.J., Plattner, G.K., Edenhofer, O., Stocker, T.F., Field, C.B., Ebi, K.L., Matschoss, P.R., 2011. The IPCC AR5 guidance note on consistent treatment of uncertainties: a common approach across the working groups. Clim. Change 108, 675–691.
Monteforti, G., Angeli, S., Petacchi, R., Minnocci, A., 2002. Ultrastructural characterization of antennal sensilla and immunocy to chemical localization of a chemosensory

protein in *Carausius morosus* Brünner (Phasmida: Phasmatidae). Arthropod Struct. Dev. 30, 195–205.
Nagnan-Le Meillour, P., Cain, A.H., Jacquin-joly, E., François, M.C., Ramachandran, S., Maida, R., Steinbrecht, R.A., 2000. Chemosensory proteins from the proboscis of *Mamestra brassicae*. Chem. Senses 25, 541–553.
Pachauri, R.K., Meyer, L., Team, T.C.W., 2014. Climate Change 2014: Synthesis Report. IPCC, Geneva, Switzerland.
Pelosi, P., Calvello, M., Ban, L., 2005. Diversity of odorant-binding proteins and chemosensory proteins in insects. Chem. Senses 30, 291–292.
Pelosi, P., Zhou, J.J., Ban, L.P., Calvello, M., 2006. Soluble proteins in insect chemical communication. Cell. Mol. Life Sci. 63, 1658–1676.
Possell, M., Hewitt, C.N., Beerling, D.J., 2005. The effects of glacial atmospheric CO_2 concentrations and climate on isoprene emissions by vascular plants. Glob. Change Biol. 11, 60–69.
Rankin, M.A., Burchsted, J.C.A., 1992. The cost of migration in insects. Annu. Rev. Entomol. 37, 533–559.
Reddy, G.V.P., Tabone, E., Smith, M.T., 2004. Mediation of host selection and oviposition behavior in the diamondback moth *Plutella xylostella* and its predator *Chrysoperla carnea* by chemical cues from cole crops. Biol. Control 29, 270–277.
Rim, H., Uefune, M., Ozawa, R., Yoneya, K., Takabayashi, J., 2017. Experience of plant infestation by the omnivorous arthropod *Nesidiocoris tenuis*, affects its subsequent responses to prey-infested plant volatiles. Biocontrol 62, 233–242.
Sachse, S., Krieger, J., 2011. Olfaction in insects—the primary processes of odor recognition and coding. Neuroforum 2, 49–60.
Schoonhoven, L.M., Jermy, T., van Loon, J.J.A., 1998. Insect-Plant Biology: From Physiology to Evolution. Cambridge University Press, pp. 217–218.
Stacey, D.A., Fellowes, M.D.E., 2002. Influence of elevated CO_2 on interspecific interactions at higher trophic levels. Global Change Biol. 8, 668–678.
Steinbrecht, R.A., Laue, M., Ziegelberger, G., 1995. Immunolocalization of pheromone-binding protein and general odorant-binding protein in olfactory sensilla of the silk moths Antheraea and Bombyx. Cell Tissue Res. 282, 203–217.
Suarez, R.K., Darveau, C.A., Welch, J.K., O'Brien, D.M., Roubik, D.W., Hochachka, P.W., 2005. Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. J. Exp. Biol. 208, 3573–3579.
Sukontason, K., Methanitikorn, R., Chaiwong, T., Kurahashi, H., Vogtsberger, R.C., Sukontason, K.L., 2007. Sensilla of the antenna and palp of *Hydrotaea chalcogaster* (Diptera: Muscidae). Micron 38, 218–223.
Sun, Y.C., Guo, H.J., Liu, Z.Y., Ge, F., 2011. The mechanisms by which elevated CO_2 affects the interaction between herbivorous insects and their host plants. Chin. J. Appl. Entomol. 48, 1123–1129.
Sun, Y.C., Guo, H., Yuan, L., Wei, J., Zhang, W., Ge, F., 2015. Plant stomatal closure improves aphid feeding under elevated CO_2. Global Change Biol. 21, 2739–2748.
Sun, Y.C., Jing, B.B., Ge, F., 2008. Response of amino acid changes in *Aphis gossypii* (Glover) to elevated CO_2 levels. J. Appl. Entomol. 133, 189–197.
Verkerk, R.H.J., Wright, D.J., 1994. Interactions between the diamondback moth, *Plutella xylostella* L. and glasshouse and outdoor-grown cabbage cultivars. Ann. Appl. Biol. 125, 477–488.
Vuorinen, T., Reddy, G.V.P., Nerg, A.M., Holopainen, J.K., 2004. Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO_2 concentration. Atmos. Environ. 38, 675–682.
Weathersbee III, A.A., Hardee, D.D., 1994. Abundance of cotton aphids (Homoptera: Aphididae) and associated biological control egents on six eotton eultivars. J. Econ. Entomol. 87, 258–265.
Wang, L., Zhang, S., Luo, J.Y., Wang, C.Y., Lu, L.M., Zhang, L.J., Zhu, X.Z., Li, C.H., Cui, J.J., 2017. Host choice of different host biotypes of cotton aphid and preliminary analysis of the mechanism. Cotton Sci. 29, 292–300.
Wang, Q.K., Zhang, M., Li, K., Zhang, D., 2012. Olfactory sensilla on antennae and maxillary palps of *Fannia hirticeps* (Stein, 1892) (Diptera: Fanniidae). Microsc. Res. Tech. 75, 1313–1320.
Xia, Y.F., Wang, G., Buscariollo, D., Jason Pitts, R., Wenger, H., Zwiebel, L.J., 2008. The molecular and cellular basis of olfactory-driven behavior in *Anopheles gambiae* larvae. PNAS 105, 6433–6438.
Xu, S.P., 2014. Cloning and Expression Analysis of Odorant Binding Proteins in *Aphis gossypii* Glover. Huazhong Agricultural University.
Yi, C.H., Chen, X.M., Shi, J.Y., Zhou, C.L., 2009. Study on changes of contents of carbohydrate and fat in *Papilio memnon* L. euring diapause stage. J. Anhui Agric. Sci. 37, 5516–5517.
You, L., Wang, G.L., Wei, H.Y., 2012. Advances in neuron transferring pathways in insects' olfactory eignals. Biol. Disaster Sci. 35, 7–11.
Zhao, J.J., Zhang, Y., Fan, D.S., Feng, J.N., 2017. Identification and expression profiling of odorant-binding proteins and chemosensory proteins of *Daktulosphaira vitifoliae* (Hemiptera: Phylloxeridae). J. Econ. Entomol. 110, 1812–1820.
Zhou, J.J., 2010. Odorant-binding proteins in insects. Vitam. Horm. 83, 241–272.